Libby Superfund Site Operable Unit 3 Standard Operating Procedure

Date: November 4, 2011 SOP DUFF-LIBBY-OU3 (Rev. 1)

Title: SAMPLING AND ANALYSIS OF DUFF FOR ASBESTOS

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
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Revision No.	Date	Reason for Revision
0	02/07/2008	--
1	11/04/2011	• Editorial changes. • Modify the recording rules for partially obscured structures. • Add caution about mass percent estimation. • Add Attachment to provide appropriate EDDs.

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized method for collection and analysis of duff samples for Libby amphibole asbestos (LA). Duff consists of the un-decomposed twigs, needles and other vegetation and the layer of partially- to fully-decomposed litter that occurs on top of the mineral soil in forested areas. This procedure will be used by the U.S. Environmental Protection Agency (USEPA), Region 8 for the Remedial Investigation work for Operable Unit 3 performed at the Libby Asbestos Superfund site.

2.0 RESPONSIBILITIES

The Field Sampling Team Leader is responsible for ensuring that all duff samples are collected in accord with this SOP. The Laboratory Director is responsible for ensuring that duff samples provided to the laboratory for evaluation by this SOP are prepared and analyzed in accord with the requirements of this SOP. It is the responsibility of the Field Sampling Team Leader and the Laboratory Director to communicate the need for any deviations from the SOP with the appropriate USEPA Region 8 Remedial Project Manager or Regional Chemist.

3. 0 EQUIPMENT

3.1 Field Equipment

- zip-top plastic bags
- sample identification labels
- GPS unit
- field log book
- field sample data sheet(s)
- ink pen
- clear packaging tape

3.2 Laboratory Equipment /Reagents

- Large aluminum trays
- Drying oven
- Large metal tray(s) (large enough for duff sample to cover bottom up to ½ in.)
- Muffle furnace
- Glass stirring rods
- Fume hood
- HEPA filtered hood
- Reagent grade or better acetone
- Reagent grade or better HCl
- Fiber-free deionized water (FDI water)
- Ultrasonic bath, producing a rate of energy deposition in the range of 0.08-0.12 MW/m^3
- Disposable plastic filter funnel apparatus

- Disposable filter funnels with straight sides [VWR # 145-0020]
- Culture dishes [VWR # 25388-581, case of 500]
- 47 mm 0.45 micron MCE or 0.4 micron PC filters
- Kim wipes or alternative paper
- zip-top plastic bags
- Glass petri dishes
- Glass microscope slides
- Low temperature plasma asher
- Vacuum evaporator (carbon coater)
- Graphite or carbon rods
- HEPA laminar flow hood
- Acetone vapor generator
- Grids
- Fine forceps
- Grid storage boxes
- Jaffe wick or sponge
- Transmission electron microscope with the following capabilities:
  - 100 Kev
  - fine probe size <250 nm
  - Energy Dispersive X-Ray Analysis (EDXA)
  - Selective Area Electron Diffraction (SAED)

4.0 METHOD SUMMARY

A duff sample is collected by hand at a selected field location and placed in a bag or container. Duff samples are prepared for analysis by high temperature ashing to remove organic matter. The residue is then analyzed for LA by transmission electron microscopy (TEM) and/or by Polarized Light Microscopy (PLM), as specified in the project-specific Sampling and Analysis Plan (SAP).

5.0 SAMPLE COLLECTION

Duff samples should be collected from the soil sampling stations specified in the project-specific SAP. At each specified sampling station, collect any fresh or partially decayed organic debris (e.g., twigs, leaves, pine needles) using a freshly-gloved hand from the soil surface within an area that is approximately 6 in. x 6 in. Care should be taken to ensure that the top layer of soil beneath the organic debris is not included in the duff material sample. Place the duff material into a large, air-tight, re-sealable plastic bag. Label the bag with the same sample identifier as the soil field sample, and place clear packaging tape over the sample identifier label.

Attachment A provides an example Field Sample Data Sheet (FSDS) for recording field information on each duff sample. [Note: in some cases, an alternative FSDS may be specified and provided in the project-specific SAP]. Note any special circumstances or conditions about the sampling location. Obtain and record the GPS coordinates of the sampling location on the FSDS form.

6.0 SAMPLE PREPARATION AND ANALYSIS

6.1 Drying and Ashing

At the laboratory, weigh and record the tare weight of a clean, dry aluminum tray of approximately quart size. Fill the aluminum tray to approximately ¾ full. The sample may be split across as many trays as may be needed, providing the applicable sample identification number is clearly marked on each tray. In addition, for tracking purposes, each tray should possess a mark to make it unique and identifiable from the other trays (e.g., Tray A, B, C). This identifier shall be recorded in the laboratory preparation logs. Each tray will be initially tared and gravimetrically tracked through the preparation process. Place the tray(s) with the sample into a drying oven. Heat to 60°C and hold at this temperature until weight stabilizes (at least 10 hours). Record the total (i.e., tray + duff) dry weight and calculate the mass of the dried duff sample by difference. If the sample was dried in more than one tray, compute the total mass of the dried duff for the sample by summation across trays.

Once duff samples are dried, they shall be ashed. Weigh and record the tare weight of one or more clean metal pans capable of withstanding the heat of a 450°C oven. Working under a hood, transfer the dried duff to the tared pan(s), place a lid on the pan and move to a muffle furnace. Ramp up the furnace from a cold start to 450°C and hold at this temperature for 18 hours or until all organic matter is removed.

Allow the pan(s) to cool. Remove the lid(s), weigh and record the mass of the pan(s) plus the ashed residue. Calculate the mass of the ashed residue in each pan by difference. If the sample was ashed in more than one pan, compute the total mass of the ashed residue for the sample by summation across pans.

Under a laminar flow hood, slowly pour the ash from each sample into individual zip-top bags. If the sample was ashed in more than one pan, all the pans for that sample should be combined into a single bag. If the ash still retains some structure, seal the bag tightly and manipulate the ash by hand to reduce it to a fine homogenous powder. Invert the bag 3-4 times to thoroughly mix the ash.

All information regarding sample preparation shall be recorded using the sample preparation log sheet, presented as Attachment B.

6.2 TEM Analysis

Acid Treatment

Remove an aliquot of approximately 0.25 g of the well-mixed ash and place into a crucible. Record the weight (measured to an accuracy of ± 0.01 g) on the sample preparation data sheet (see Attachment B). To the ashed residue in the crucible, add just enough FDI water (approximately 1-2 mL) to cover the surface of the residue. Slowly add concentrated HCl to the wetted ash (approximately 10-20 mL). Typically, a visible effervescing is observed. Add the HCl slowly to

keep this reaction controlled. A small glass stirring rod is useful at this point to gently stir the ash and expose all material to the acid.

If there is no further visible reaction after 3-5 minutes, proceed to the next step. If bubbling is still occurring, continue observation and gentle stirring for up to an additional 5 minutes.

Dilute the sample by adding FDI water directly to the crucible (approximately 20 mL) using a squirt bottle. Pour the sample into an unused disposable 100 mL specimen container with lid. Rinse out any remaining residue from the crucible into the specimen container. Do not exceed 100 mL total volume. Bring the total volume to 100 mL with DI water.

Cap the specimen cup and agitate the sample by inversion 5 or 6 times. Loosen the cap slightly and sonicate for 2 minutes. After sonication, tighten the cap and then dry the exterior of the specimen container with a laboratory wipe.

Filtration

Agitate the sample by inversion 5 or 6 times. Withdraw an initial aliquot of 0.1 to 1 mL of sonicated sample. Transfer this aliquot into a new disposable specimen container with lid. Bring the volume up to approximately 100 mL with FDI water. Cap and agitate by inversion (5 or 6 times).

Filter this entire volume onto a 47 mm mixed cellulose ester (MCE) filter with 0.4 um pore size.

If the filter appears overloaded (overall particulate level > 20%), repeat the process above, selecting a smaller aliquot volume, as suggested by the degree of overloading. Conversely, if the filter looks too lightly loaded, filter a larger aliquot.

After filtration, transfer the filter membranes to individual disposable labeled Petri dishes with lids. With Petri dish covers ajar, gently air dry the filters in a HEPA-protected environment.

TEM Examination

Prepare 3 grids for TEM analysis as detailed in International Organization for Standardization (ISO) TEM method 10312, also known as ISO 10312:1995(E). Utilize 2 grids for analysis, archiving the third grid in case of problems. After analysis, archive all three grids for potential future reanalysis.

Counting and Recording Rules

Examine the grids using TEM in basic accordance with ISO 10312 and all relevant Libby site-specific modifications, including the most recent version of LB-000016, LB-000019, LB-000028, LB-000029, LB-000030, LB-000053, and LB-000066. However, this SOP does differ from ISO 10312 in the recording rules used for partially obscured structures (ISO 10312, Section C.4.8).

All fibrous amphibole structures that have appropriate Selective Area Electron Diffraction (SAED) patterns and Energy Dispersive X-Ray Analysis (EDXA) spectra, and having length greater than or equal to 0.5 um and an aspect ratio (length: width) ≥ 3:1, will be recorded. Structures should be recorded using ISO 10312 structure reporting methodology, with the following SOP-specific modification:

- For partially obscured structures, the proportion of the structure that is obscured by particulates shall be used as the basis for determining the appropriate recording methodology.
    - If the obscured length could not possibly be more than one-third of the total length, the structure should be recorded in accordance with the ISO 10312 recording procedures for disperse clusters and matrices. That is, the primary complex structure (e.g., MD, CD) would be recorded with the component structure(s) (e.g., MF, CF) recorded separately. The assigned length for the partially obscured component structure shall be equal to the visible length plus the maximum possible contribution from the obscured portion.
    - If the obscured length could be more than one-third of the total length, the structure should be recorded using the same procedure as discussed above, except that the structure type of the component structure(s) should be recorded with an "O" suffix (i.e., MFO, MBO). Figure 1 presents two examples that illustrate these structure recording rules.

Raw structure data should be recorded on the Libby site-specific laboratory bench sheets and electronic data deliverable (EDD) spreadsheet for TEM analysis of duff samples (see Attachment C). Data recording for chrysotile (if observed) is not required.

Stopping Rules

Examine a minimum of 2 grid openings in each of 2 grids. Continue examining grid openings until one of the following occurs:

- The target sensitivity is achieved.
- A total of 50 or more LA structures are observed. In this case, counting may cease after completion of the grid opening that contains the 50th LA structure.
- A total of 100 grid openings are counted without reaching the target sensitivity or observing 50 LA structures. In this event, the analysis should stop after completion of the 100th grid opening.

The target analytical sensitivity for sample analysis should be specified in the SAP. In the absence of a project-specific target sensitivity, the default analytical sensitivity should be 1E+07 (grams dry weight)$^{-1}$. The analytical sensitivity is calculated using the following equation:

$$S = \frac{EFA}{GO \cdot Ago \cdot Mass \cdot F}$$

where:

S	=	Sensitivity (1/grams dry weight)
EFA	=	Effective filter area (mm^2)
GO	=	Number of grid openings counted
Ago	=	Area of one grid opening (mm^2)
Mass	=	Mass of the dried (but not ashed) duff sample (g)
F	=	Fraction of the dried duff sample applied to the filter

TEM Data Deliverable

All data on the number, type, and size of LA structures observed during TEM analysis in the laboratory will be transmitted as an electronic data deliverable (EDD) using the most recent version of the spreadsheet developed for this purpose (see Attachment C). The results for each sample will be expressed in terms of LA structures per gram duff (dry weight). If desired, the raw structure data can be utilized to calculate an estimate of mass percent (grams of LA per 100 grams of duff dry weight). However, estimates of mass percent derived using this approach should be regarded as highly uncertain, and the true mass percent may be either higher or lower than the reported value.

6.3 PLM Analysis

If analysis by PLM is called for in the project-specific SAP, the analysis will be performed on an aliquot of the ashed and homogenized residue using method PLM-VE as detailed in the most recent version of SOP SRC-LIBBY-03. PLM-VE is a semi-quantitative analytical method for asbestos that utilizes Libby-specific reference materials to allow assignment of samples into one of four mass percent "bins", as follows:

- Bin A (ND): non-detect
- Bin B1 (Trace): LA detected at levels lower than the 0.2% reference material
- Bin B2 (<1%): LA detected at levels lower than the 1% reference material but higher than the 0.2% reference material
- Bin C: LA detected at levels greater than or equal to 1%

A potential limitation to this approach is that the site-specific reference materials are based on LA in soil, not LA in ashed residue. This may introduce additional uncertainty into the results, but no reference materials based on ashed residue are presently available.

PLM-VE results will be recorded using the most recent version of the Libby site-specific EDD spreadsheet for PLM-VE analysis (see Attachment C).

7.0 QUALITY ASSURANCE

7.1 Field-Based Quality Assurance

Field Duplicates

Field duplicate duff samples will be collected at a frequency specified in the project-specific SAP. In the absence of such specification, the rate should be no less than 5%. Each field duplicate should be collected from a location close to the primary sample, and from an area of approximately equal size. Field duplicate samples should be labeled with a unique identifier. Sample details should be recorded on the appropriate FSDS, including the unique identifier of the "parent" field sample. Field duplicates are used to evaluate the sampling and analysis variability across duff samples. Unless indicated differently in the project-specific SAP, samples will not be qualified purely as a result of the difference between measured values between original and duplicate pairs.

7.2 Laboratory-Based Quality Assurance for TEM Analyses

Drying Blanks

For the purposes of this analysis, a drying blank will consist of one clean aluminum pan placed empty into the drying oven along with pans containing field samples of duff. After drying the duff samples, the clean tray will be removed and the surface will be rinsed with about 100 mL of FDI water into a clean container, which in turn will be filtered and prepared for TEM analysis. Detection of fibers on the drying blank filter will be taken as an indication of potential cross-contamination during drying.

Drying blanks should be prepared at a rate specified in the project-specific SAP. In the absence of a project-specific specification, drying blanks should be prepared at a rate of one per day that drying of samples is occurring. Unless indicated differently in the project-specific SAP, if the drying blank reports LA fibers, all samples in that drying batch will be assigned a qualifier to indicate the potential for cross-contamination.

Laboratory Blanks

A laboratory blank is a filter that is prepared by processing a clean crucible in the same way that a duff sample is prepared. That is, a clean crucible is treated by addition of FDI water and HCl, as described above. The contents of the crucible are then rinsed out, diluted to 100 mL, and an aliquot at least as large as the highest volume aliquot for the sample set is removed and used to prepare a filter for TEM examination. This type of blank is intended to indicate if contamination is occurring at any stage of the sample preparation procedure.

Laboratory blanks should be prepared at a rate specified in the project-specific SAP. In the absence of a project-specific specification, laboratory blanks should be prepared at a rate of 3%. Unless indicated differently in the project-specific SAP, if the laboratory blank reports LA fibers, all samples in that analytical batch will require re-preparation.

Filtration Blanks

A filtration blank is a clean filter that is prepared by passing 100 mL of laboratory FDI water through it. The purpose of this type of blank is to ensure that the filters are not contaminated in the laboratory, and that fluids used for diluting and processing samples are fiber-free.

Filtration blanks should be prepared at a rate specified in the project-specific SAP. In the absence of a project-specific specification, filtration blanks should be prepared at a rate of 2%. Unless indicated differently in the project-specific SAP, if the laboratory blank reports LA fibers, all samples in that analytical batch will require re-preparation.

Laboratory Duplicates

Laboratory duplicates will be prepared by applying a second aliquot of ashed residue suspension to a new filter, which is then prepared and analyzed in the same fashion as the original filter. The frequency of laboratory duplicates should be specified in the project-specific SAP. In the absence of such specification, the rate should be no less than 5%. Unless indicated differently in the project-specific SAP, samples will not be qualified purely as a result of the difference between measured values between original and duplicate pairs.

Recounts

The precision of TEM sample results should be evaluated by recounting selected grid openings in accordance with the requirements specified in the most recent version of LB-000029.

7.3 Laboratory-Based Quality Assurance for PLM-VE Analyses

Laboratory Duplicates

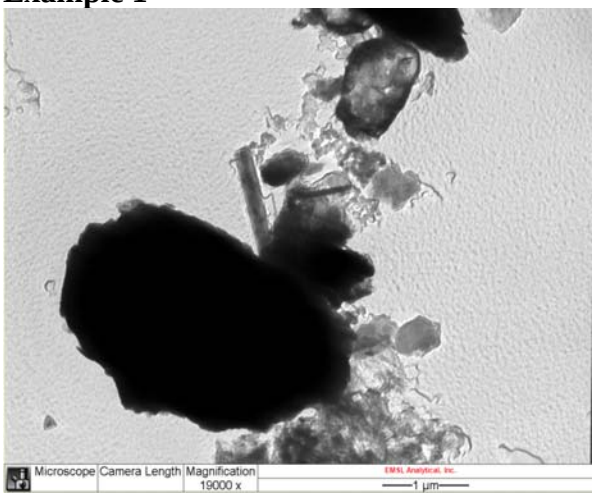
Laboratory duplicate PLM-VE analyses will be prepared by examining a second aliquot of ashed and homogenized residue. The frequency of laboratory duplicates should be specified in the project-specific SAP. In the absence of such specification, the rate should be no less than 5%. Unless indicated differently in the project-specific SAP, samples will not be qualified purely as a result of the difference between measured values between original and duplicate pairs.

8.0 REFERENCES

International Organization for Standardization. 1995. *Ambient Air – Determination of asbestos fibres – Direct-transfer transmission electron microscopy method.* ISO 10312:1995(E).

FIGURE 1
ILLUSTRATION OF STRUCTURE RECORDING RULES
FOR PARTIALLY OBSCURED STRUCTURES

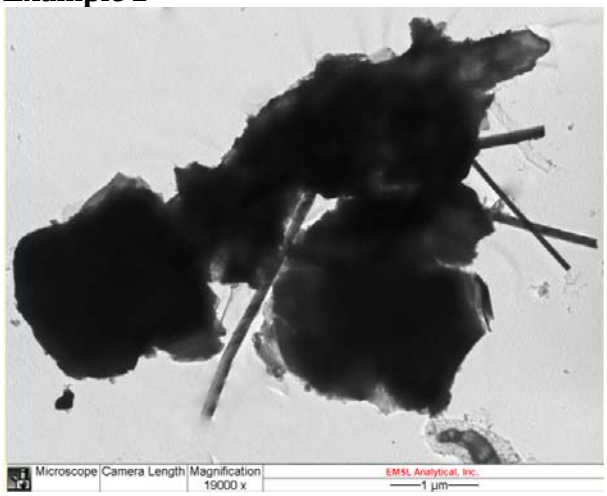
Example 1



Count as 1 disperse matrix consisting of 1 component fiber that is less than 5 um in length.

Record as MD10, followed by 1 fiber recorded as MFO. Recorded length for the MFO should be equal to the observed length plus the maximum possible length of the obscured portion.

Example 2



Count as 1 disperse matrix consisting of 4 component fibers that are all less than 5 um in length.

Record as MD40, followed by 4 fibers recorded as MFO. Recorded length for each MFO should be equal to the observed length plus the maximum possible length of the obscured portion.

Photos courtesy of EMSL Analytical, Inc.

ATTACHMENT A

FIELD SAMPLE DATA SHEET (FSDS) FOR DUFF

LIBBY FIELD SAMPLE DATA SHEET (FSDS) rev0
DUFF

Field Logbook No: _____ Page No: _____
Station ID: _____ Sampling Date: _____
GPS Coordinate System: _____ Elevation Coordinate System: _____
X coordinate:_____ Y coordinate:_____ Elevation:_____
Sampling Team: _____ Sampler Initials: _____
Station Comments: _____
_____

Data Item	Sample 1	Sample 2	Sample 3
Index ID (place pre-printed label in field provided)			
Sample Time (hh:mm)			
Sample Type (circle one):	Grab Composite # of Composites: _____	Grab Composite # of Composites: _____	Grab Composite # of Composites: _____
Field QC Type (circle one):	FS (field sample) FD (field duplicate) For FD, Parent ID: _____	FS (field sample) FD (field duplicate) For FD, Parent ID: _____	FS (field sample) FD (field duplicate) For FD, Parent ID: _____
Field Comments:			
Entered by (Provide initials):		**Validated by (Provide initials):**	

ATTACHMENT B

DUFF PREPARATION SAMPLE DATA SHEET (PSDS)

[provided electronically in "DUFF PSDS rev 1.xls"]

LIBBY DUFF PREPARATION SAMPLE DATA SHEET (PSDS)

PAGE _____ of _____

Laboratory Name: ____________________ Lab Job No.: ____________________ Lab QC Batch No.: ____________________ SOP: DUFF-LIBBY-OU3 (Rev 1)

Preparation by: ____________________ Preparation Date: ____________________

Drying Oven Temp. (^oC): ____________ Muffle Furnace Temp. (^oC): ____________ HCL Reagent Tracking No: ____________________

	SAMPLE INFORMATION			DRYING						ASHING				FILTER PREP			Notes
	Index ID	Lab Sample ID	Mass (g), as received	Tray ID(s) used in drying	Tray weight (g)	Mass (g), during drying [tray + sample] Check 1	Check 2	Check 3	Mass (g), after drying [sample only]	Pan ID(s) used in ashing	Pan weight (g)	Mass (g), after ashing [pan + sample]	Mass (g), after ashing [sample only]	Mass of ash (g) taken for analysis	Volume of HCl added (mL)	Aliquot volume (mL)	
Example	X-12345	026589	500.3	A	5.71	63.12	55.90	55.84	50.13	A	15.87	36.98	21.11	0.26	15.7	1.0	
Example	↓	↓	↓	B	4.99	70.56	63.02	63.11	58.12	B	16.20	44.05	27.85				
Example	↓	↓	↓	C	5.23	89.63	71.85	72.03	66.8								

Note: All mass measurements should be recorded to an accuracy of ± 0.01 g.

QA Check by: ____________________ Date: ____________

ATTACHMENT C

ELECTRONIC DATA DELIVERABLE (EDD) SPREADSHEETS

[contact EPA to obtain copies of the most recent version of the EDDs]

Date: November 4, 2011 SOP TREE-LIBBY-OU3 (Rev. 2)

Title: SAMPLING AND ANALYSIS OF TREE BARK FOR ASBESTOS

APPROVALS:

TEAM MEMBER	SIGNATURE/TITLE	DATE
EPA Remedial Project Manager	Christina Progess, USEPA RPM	11/4/11
SOP Author	Lynn Woodbury, CDM	11/4/11

Revision Number	Date	Reason for Revision
0	09/26/2007	--
1	11/20/2007	Modify procedure for sample preparation based on results of pilot-scale laboratory tests.
2	11/04/2011	• Editorial changes. • Modify the recording rules for partially obscured structures. • Add Attachments to provide example FSDS and appropriate EDD.

1.0 PURPOSE

The purpose of this Standard Operating Procedure (SOP) is to provide a standardized method for collection and analysis of tree bark samples for asbestos. This procedure will be used by U.S. Environmental Protection Agency (USEPA), Region 8 for the Remedial Investigation work for Operable Unit 3 performed at the Libby Asbestos Superfund site.

2.0 RESPONSIBILITIES

The Field Sampling Team Leader is responsible for ensuring that all bark samples are collected in accord with this SOP. The Laboratory Director is responsible for ensuring that bark samples provided to the laboratory for evaluation by this SOP are prepared and analyzed in accord with the requirements of this SOP. It is the responsibility of the Field Sampling Team Leader and the Laboratory Director to communicate the need for any deviations from the SOP with the appropriate USEPA Region 8 Remedial Project Manager or Regional Chemist.

3. 0 EQUIPMENT

3.1 Field Equipment

- hole saw (2-inch diameter)
- battery-powered drill
- ½ inch chisel
- flathead screwdriver
- aerosol hair spray
- zip-top plastic bags
- sample identification tags
- decontamination supplies
- trash bag
- GPS unit
- digital camera
- flagging tape or metal identification tag
- field log book
- field sample data sheet(s) for tree bark
- increment boring device (e.g., Hagloff)
- plastic sheath for age core
- ink pen
- clear packaging tape

3.2 Laboratory Equipment /Reagents

- Disposable Filter funnels with Straight sides. VWR # 145-0020
- Culture Dishes. VWR # 25388-581 (case of 500)
- 47 mm 0.45 micron mixed cellulose ester (MCE) filters
- Glass Petri Dishes

- Glass microscope slides
- Low Temperature Plasma Asher
- Vacuum Evaporator (Carbon Coater)
- Graphite or Carbon rods
- HEPA Laminar Flow Hood
- Acetone Vapor Generator
- Grids
- Fine Forceps
- Grid Clips and Grid Storage Boxes
- Jaffe Wick or Sponge
- Kim wipes or alternative paper
- Transmission Electron Microscope with the following capabilities:
  - 100 Kev
  - fine probe size <250 nm
  - elemental Chemistry via X-Ray Detector
- Large ceramic crucibles (approx. 50 ml capacity or greater)
- Glass stirring rods
- Fumehood
- HEPA filtered Hood
- Ultrasonic Bath producing a rate of energy deposition in the range of 0.08-0.12 MW/m^3
- Disposable plastic filter funnel apparatus
- Reagent Grade or better Acetone
- Reagent Grade or better HCl

4.0 METHOD SUMMARY

One or more tree bark samples are obtained from selected trees by using a 2-inch hole saw to cut a circular ring in the bark, following by cutting/prying the circular piece of bark from the tree using a sharp chisel. The area to be sampled is sprayed with hair spray prior to sample collection in order to minimize the potential for loss of fibers from the tree bark. In some cases, a core may be obtained from the tree in order to allow verification of the age of the tree.

Tree bark samples are prepared for analysis by high temperature ashing to remove organic matter. The residue is then treated with HCl to dissolve any salts or carbonate component that may be present and applied to a filter which is examined for asbestos using transmission electron microscopy (TEM).

5.0 SAMPLE COLLECTION

Bark samples should be collected from the sampling stations specified in the Sampling and Analysis Plan (SAP). At each specified sampling station, sample collection should be performed as follows:

5.1 Select Tree

The species and size of tree selected for sampling should be specified in the project-specific sampling and analysis plan. In the absence of specification, the tree selected for sampling should be a Douglas fir (*Pseudotsuga menziesii*) with a diameter (caliper) of about 8-10 inches. If there are multiple trees that meet these requirements in the vicinity of the sampling station, preference should be given to trees with rough bark, and trees that are in open areas.

5.2 Bark Collection

Collect the bark sample from the side of tree facing the mine and from a height of 4-5 feet above the ground.

Steps:

1. Spray the bark collection area with aerosol hairspray and allow to dry.
2. Use a 2-inch diameter hole saw and a battery-powered electric drill to cut a circle in the tree bark. Continue cutting until sawdust changes from red to cream, which indicates that the cambium has been reached (about ½ inch deep).
3. Using a sharp ½-inch metal chisel or flathead screwdriver, cut or pry the circular bark sample off the tree, attempting to maintain the sample in one piece.
4. Place the bark sample in a plastic zip-top bag.
5. Label the bag with a unique sample identifier.
6. Place clear packaging tape over the sample identifier label.

5.3 Tree Age Core Collection

At locations where an age core is to be collected (as specified in the project-specific sampling and analysis plan), collect a core from the tree using a Hagloff manual increment borer or similar device. Place the core in a plastic straw. Crimp and tape the ends of the straw, and label the straw with the same sample identifier as the bark field sample. Place the straw into a zip-top bag. Label the bag with the same sample identifier as the bark field sample, and place clear packaging tape over the sample identifier label.

5.4 Field Documentation

Complete the Tree Bark Field Sample Data Sheet (FSDS) form (see Attachment A). Measure and record the diameter of the tree. Obtain and record the GPS coordinates of the tree on the Tree Bark FSDS. Mark the tree with flagging tape or a metal identification tag.

5.5 Equipment Decontamination

If dedicated sample equipment is not used, after each sample collection, manually remove any fibrous debris from the hole saw teeth. If resin or pitch is present, use WD40 to clear saw of any residue. Thoroughly clean all collection equipment with isopropyl alcohol wipes. Dry the sampling equipment using paper towels. Any spent wipes, paper towels, or other decontamination waste materials must be disposed or stored properly as investigation-derived waste.

6.0 SAMPLE PREPARATION AND ANALYSIS

6.1 Tree Bark Preparation

Drying and Ashing

Measure and record the diameter and the thickness of the tree bark sample to an accuracy of ± 2 mm (about 1/16 of an inch).

Weigh and record the tare weight of a clean crucible. Add the entire tree bark core to the crucible. Place the crucible with bark sample in a drying oven. Heat to 80°C and hold at this temperature until weight stabilizes (at least 6 hours). Record the final weight and calculate the mass of the dried tree bark sample by difference.

Place a lid on the crucible and transfer to a muffle furnace. Ramp up the furnace from a cold start to 450°C; hold at this temperature for 18 hours or until all organic matter is removed. Allow the crucible to cool. Remove crucible lid and weigh and record the mass of the crucible plus ashed residue. Calculate the mass of the ashed residue by difference.

Acid Treatment

To the ashed residue, add just enough filtered and deionized (FDI) water (approximately 1-2 mL) to cover the surface of the residue. Slowly add approximately 10-20 mL concentrated HCl to the wetted ash. Typically a visible effervescing is observed. Add the HCl slowly to keep this reaction controlled. A small glass stirring rod is useful at this point to gently stir the ash and expose all material to the acid.

If there is no further visible reaction after 3-5 minutes, proceed to the next step. If bubbling is still occurring, continue observation and gentle stirring for up to an additional 5 minutes.

Dilute the sample by adding FDI water directly to the crucible (approximately 20 mL) using a squirt bottle. Pour the sample into an unused disposable 100 mL specimen container with lid. Rinse out any remaining residue from the crucible into the specimen container. Do not exceed 100 mL total volume. Bring the total volume to 100 ml with FDI water.

Cap the specimen jar and agitate the sample by inversion 5 or 6 times. Loosen the cap slightly and sonicate for 2 minutes. After sonication, tighten the cap and then dry the exterior of the specimen container with kim wipe or equivalent.

Filtration

Agitate the sample by inversion 5 or 6 times. Withdraw an initial aliquot of 5 to 20 mL of sonicated sample. Transfer this aliquot into a new disposable specimen container with lid. Bring the volume up to approximately 100 mL with FDI water. Cap and agitate by inversion (5 or 6 times).

Filter this entire volume onto a 47 mm mixed cellulose ester (MCE) filter with 0.4 um pore size.

If the filter appears overloaded (overall particulate level > 20%), repeat the process above, selecting a smaller aliquot volume, as suggested by the degree of overloading. Likewise, if the filter looks too lightly loaded, remove and filter a larger aliquot.

Transfer the filter membranes to individual disposable labeled Petri dishes with lids. With the Petri dish covers ajar, dry the filters by air drying.

6.2 TEM Examination

Prepare 3 grids for TEM analysis as detailed in International Organization for Standardization (ISO) TEM method 10312, also known as ISO 10312:1995(E). Utilize 2 grids for analysis, archiving the third grid in case of problems. After analysis, archive all three grids for potential future reanalysis.

Counting rules

Counting and Recording Rules

Examine the grids using TEM in basic accordance with ISO 10312 and all relevant Libby site-specific modifications, including the most recent version of LB-000016, LB-000019, LB-000028, LB-000029, LB-000030, LB-000053, and LB-000066. However, this SOP does differ from ISO 10312 in the recording rules used for partially obscured structures (ISO 10312, Section C.4.8).

All fibrous amphibole structures that have appropriate Selective Area Electron Diffraction (SAED) patterns and Energy Dispersive X-Ray Analysis (EDXA) spectra, and having length greater than or equal to 0.5 um and an aspect ratio (length: width) ≥ 3:1, will be recorded. Structures should be recorded using ISO 10312 structure reporting methodology, with the following SOP-specific modification:

- For partially obscured structures, the proportion of the structure that is obscured by particulates shall be used as the basis for determining the appropriate recording methodology.
    - If the obscured length could not possibly be more than one-third of the total length, the structure should be recorded in accordance with the ISO 10312 recording procedures for disperse clusters and matrices. That is, the primary complex structure (e.g., MD, CD) would be recorded with the component structure(s) (e.g., MF, CF) recorded separately. The assigned length for the partially obscured component structure shall be equal to the visible length plus the maximum possible contribution from the obscured portion.
    - If the obscured length could be more than one-third of the total length, the structure should be recorded using the same procedure as discussed above, except that the structure type of the component structure(s) should be recorded with an “O” suffix

(i.e., MFO, MBO). Figure 1 presents two examples that illustrate these structure recording rules.

Raw structure data should be recorded on the Libby site-specific laboratory bench sheets and electronic data deliverable (EDD) spreadsheet for TEM analysis of tree bark samples (see Attachment B). Data recording for chrysotile (if observed) is not required.

Stopping Rules

Examine a minimum of 2 grid openings in each of 2 grids. Continue examining grid openings until one of the following occurs:

- The target sensitivity is achieved.
- A total of 50 or more LA structures are observed. In this case, counting may cease after completion of the grid opening that contains the 50th LA structure.
- A total of 100 grid openings are counted without reaching the target sensitivity or observing 50 LA structures. In this event, the laboratory should contact EPA asking for direction.

The target analytical sensitivity for sample analysis should be specified in the SAP. In the absence of such specification, the target sensitivity should be no higher than 100,000 cm^{-2}. The analytical sensitivity is calculated using the following equation:

$$S = \frac{EFA}{GO \cdot Ago \cdot A \cdot F}$$

where:

S	=	Sensitivity (cm^{-2})
EFA	=	Effective filter area (mm^2)
GO	=	Number of grid openings counted
Ago	=	Area of one grid opening (mm^2)
A	=	Area of tree bark sample being analyzed (cm^2)
F	=	Fraction of original sample deposited on the filter

6.3 Electronic Data Deliverable

All data on the number, type, and size of LA structures observed during TEM analysis in the laboratory will be transmitted as an electronic data deliverable (EDD) using the most recent version of the spreadsheet developed for this purpose (see Attachment B). The results for each sample will be expressed in terms of surficial loading (LA structures per cm^2 of tree bark).

6.4 Analysis of Core Sample

The age of the tree will be determined from the core sample in accord with the method of Phipps (1985).

7.0 QUALITY ASSURANCE

7.1 Field-Based Quality Assurance

Field Duplicates

Field duplicate tree bark samples will be collected at a frequency specified in the SAP. Each field duplicate should be collected from the same tree at a location no further than 6 inches away from the original bark sample. In the absence of such specification, the rate should be no less than 5%. Field duplicate samples should be labeled with a unique identifier. Sample details should be recorded on the Tree Bark FSDS, including the unique identifier of the "parent" field sample.

Equipment Rinsates

If dedicated sampling equipment is not utilized, equipment rinsates should be collected after decontamination of field equipment as described above. The decontaminated equipment (hole saw, chisel) should be rinsed with about 25 mL filtered and deionized water into a glass container. The frequency of rinsate collection should be specified in the SAP. In the absence of such specification, one rinsate sample should be collected per sampling team per day. Equipment rinsate samples should be labeled with a unique identifier. Sample details should be recorded on the Surface Water FSDS.

7.2 Laboratory-Based Quality Assurance

Laboratory Blanks

A laboratory blank is a filter that is prepared by processing a clean crucible in the same way that a bark sample is prepared. That is, a clean crucible in placed in the oven (with the sample set) at the same time that tree-bark samples are undergoing ashing. After ashing, the blank crucible is treated by addition of water and HCl, as described above. The contents of the crucible are then rinsed out, diluted to 100 mL, and an aliquot at least as large as the highest volume aliquot for the sample set is removed and used to prepare a filter for TEM examination. This type of blank is intended to indicate if contamination is occurring at any stage of the sample preparation procedure.

Laboratory blanks should be prepared at a rate specified in the project-specific sampling and analysis plan. In the absence of a project-specific specification, laboratory blanks should be prepared at a rate of 3%.

Filtration Blanks

A filtration blank is a clean filter that is prepared by passing 100 mL of laboratory FDI water through it. The purpose of this type of blank is to ensure that the filters are not contaminated in the laboratory, and that fluids used for diluting and processing samples are fiber-free.

Filtration blanks should be prepared at a rate specified in the project-specific sampling and analysis plan. In the absence of a project-specific specification, filtration blanks should be prepared at a rate of 2%.

Laboratory Duplicates

Laboratory duplicates will be prepared by applying a second aliquot of ashed residue suspension to a new filter, which is then prepared and analyzed in the same fashion as the original filter. The frequency of laboratory duplicates should be specified in the SAP. In the absence of such specification, the rate should be no less than 5%. Laboratory duplicates should be recorded using the appropriate laboratory quality control field in the TEM EDD spreadsheet.

Recounts

The precision of TEM sample results should be evaluated by recounting selected grid openings in accord with the requirements specified in the most recent version of LB-000029.

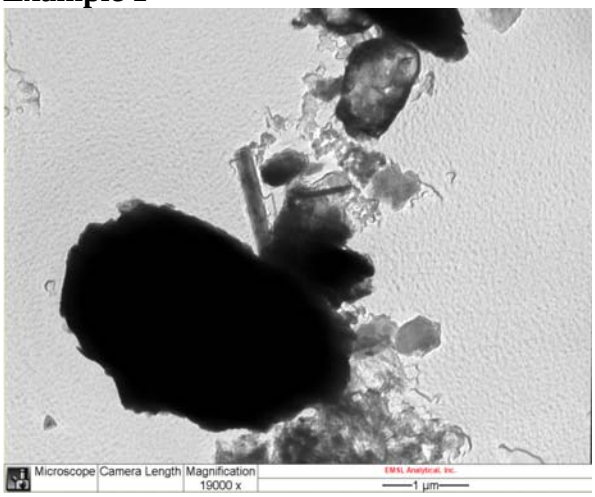
8.0 REFERENCES

International Organization for Standardization. 1995. *Ambient Air – Determination of asbestos fibres – Direct-transfer transmission electron microscopy method.* ISO 10312:1995(E).

Phipps, R.L. 1985. *Collecting, Preparing, Cross-dating and Measuring Tree Increment Cores.* U.S. Geological Survey Water Resources Investigations Report 85-4148.

FIGURE 1
ILLUSTRATION OF STRUCTURE RECORDING RULES
FOR PARTIALLY OBSCURED STRUCTURES

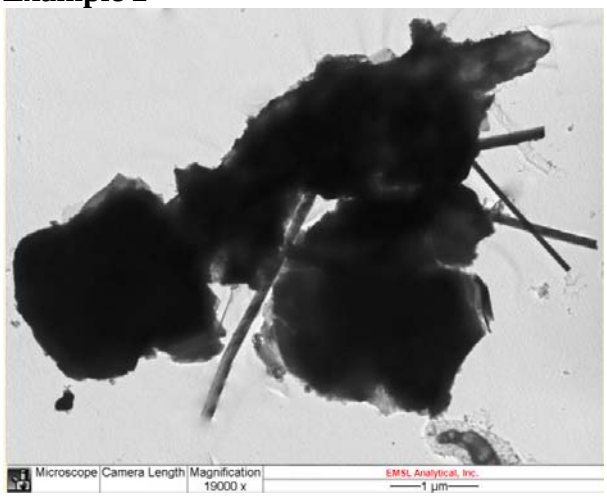
Example 1



Count as 1 disperse matrix consisting of 1 component fiber that is less than 5 um in length.

Record as MD10, followed by 1 fiber recorded as MFO. Recorded length for the MFO should be equal to the observed length plus the maximum possible length of the obscured portion.

Example 2



Count as 1 disperse matrix consisting of 4 component fibers that are all less than 5 um in length.

Record as MD40, followed by 4 fibers recorded as MFO. Recorded length for each MFO should be equal to the observed length plus the maximum possible length of the obscured portion.

Photos courtesy of EMSL Analytical, Inc.

ATTACHMENT A

FIELD SAMPLE DATA SHEET (FSDS) FOR TREE BARK

LIBBY FIELD SAMPLE DATA SHEET (FSDS)
TREE BARK

Field Logbook No: _____ Page No: _____

Station ID: _____ Sampling Date: _____

GPS Coordinate System: _____

X coord: _____ Y coord: _____ Elevation: _____m

Sampling Team: _____ Sampler Initials: _____

Station Comments: _____
_____
_____

Index ID:	**Field QC Type (circle one):** FS (field sample) FD (field duplicate) For FD, Parent ID: _____	**Sample Area (cm^2):** _____	**Tree Species:** _____ **Collection Height (ft):** _____	**Age Core Collected? (circle one):** Y N
Index ID:	**Field QC Type (circle one):** FS (field sample) FD (field duplicate) For FD, Parent ID: _____	**Sample Area (cm^2):** _____	**Diameter* (in):** _____	
Field Comments:				
Entered by (Provide initials):		**Validated by (Provide initials):**		

*Measured with "D-tape"

ATTACHMENT B

ELECTRONIC DATA DELIVERABLE (EDD) SPREADSHEET

[contact EPA to obtain copy of the most recent version of the EDD]